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     (FILE 'HOME' ENTERED AT 11:43:16 ON 15 SEP 2003)

     FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
     ENTERED AT 11:44:15 ON 15 SEP 2003
L1       1706346 S MUTANT OR MUTATION OR DELETION
L2          1181 S L1 AND AMPLICON
L3             0 S L2 AND SHORT PCR
L4            50 S L1 AND SHORT PCR
L5            12 S L4 AND AMPLICON#
L6            20 DUP REM L4 (30 DUPLICATES REMOVED)
L7             3 DUP REM L5 (9 DUPLICATES REMOVED)
L8             1 S L2 AND (CLEAVAGE  REAGENT OR DNAZYME)

     FILE 'STNGUIDE' ENTERED AT 12:07:12 ON 15 SEP 2003

     FILE 'CAPLUS, MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT'
     ENTERED AT 12:11:59 ON 15 SEP 2003
L9           168 S SHORT PCR
L10           18 S L9 AND MITOCHONDR?
L11            8 DUP REM L10 (10 DUPLICATES REMOVED)
L12           59 DUP REM L9 (109 DUPLICATES REMOVED)
L13            3 S L12 AND AMPLICON

     FILE 'STNGUIDE' ENTERED AT 12:17:14 ON 15 SEP 2003

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Mutational scanning of **mitochondrial** DNA by two-dimensional electrophoresis

AUTHOR(S): Van Orsouw, Nathalie J.; Zhang, Xiaomin; Wei, Jeanne Y.; Johns, Donald R.; Vijg, Jan

CORPORATE SOURCE: Gerontol. Div., Dep. Med., Beth Israel Deaconess Med. Cent. and Harvard Med. Sch., Harvard Inst. Med., Boston, MA, 02115, USA

SOURCE: Genomics (1998), 52(1), 27-36
CODEN: GNMCEP; ISSN: 0888-7543

PUBLISHER: Academic Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB An expedient, accurate, and cost-efficient test was developed to scan crit. regions of the **mitochondrial** genome for all possible mutations by two-dimensional DNA electrophoresis. The test involves a two-step multiplex PCR amplification: a long-distance PCR to amplify almost the entire **mitochondrial** genome, which then serves as template for the amplification of 25 **short PCR** fragments in two multiplex groups corresponding to regions implicated in human diseases. The mixt. of fragments was subsequently subjected to two-dimensional electrophoretic sepn., first by size in a nondenaturant polyacrylamide gel and then on the basis of basepair sequence in a denaturing gradient polyacrylamide gel. This latter process of denaturing gradient gel electrophoresis is a most accurate form of mutation detection on the basis of differences in melting behavior of mutant and wildtype fragments. Evaluation of the method using samples with known homoplasmic and heteroplasmic mutations, as well as CEPH pedigrees to study segregation of polymorphic variants, indicated a very high accuracy; none of the previously identified mutations and polymorphisms escaped detection, and no erroneous segregation patterns of polymorphic variants were obsd. In addn., two variants were found to be novel mutations when analyzed by sequence anal. One of these novel mutations was a heteroplastic mutation in the COXIII gene that was found to segregate to homoplasmy in the next generation. Heteroplasmic mutations as low as 1% of mtDNA could still be detected. (c) 1998 Academic Press.

L7 ANSWER 1 OF 3 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-08727 BIOTECHDS
TITLE: Detecting **mutant** nucleic acid, preferably for detecting the presence of a **deletion**, comprises contacting a sample containing nucleic acids with **mutant** primers, and amplifying under short polymerase chain reaction conditions;
DNA primer for **deletion mutation** detection
AUTHOR: SUTHERLAND J W
PATENT ASSIGNEE: ORTHO CLINICAL DIAGNOSTICS INC
PATENT INFO: EP 1266970 18 Dec 2002
APPLICATION INFO: EP 2002-254040 11 Jun 2002
PRIORITY INFO: US 2001-877748 11 Jun 2001; US 2001-877748 11 Jun 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-177324 [18]
AN 2003-08727 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Detecting **mutant** nucleic acid comprises: (a) contacting a sample comprising nucleic acids with **mutant** primers;(b) amplifying the product under short polymerase chain reaction (PCR) conditions; and (c) identifying the presence of **amplicons**, which indicates the presence of nucleic acid **deletion** sequences.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) quantifying nucleic acids having **deletion** sequences; and (2) a kit for detecting or quantifying the occurrence of nucleic acid **deletion** sequences, comprising **mutant** PCR primers.

BIOTECHNOLOGY - Preferred Method: Detecting a nucleic acid having a **deletion** comprises: (a) obtaining a sample comprising nucleic acids; (b) dividing the sample into 2 aliquots, each suspected of containing a mixture of **mutant** DNA and wild type DNA; (c) contacting the first aliquot with a cleavage reagent to form a mixture; (d) contacting the mixture with a forward primer complementary to a priming site upstream of the **deletion** sequence; (e) to the mixture of (d), adding a reverse primer complementary to the downstream zone of the **mutant** DNA and each of 4 different nucleoside triphosphates as well as a DNA polymerase under conditions so that only the **mutant** DNA is amplified; (f) contacting the second aliquot with a forward primer complementary to a priming site upstream of the **deletion** sequence and a reverse primer complementary to a priming site within the **deletion** sequence; (g) to the mixture in (f), adding 4 different nucleoside triphosphates, and a DNA polymerase under conditions so that the DNA is amplified; and (h) detecting the presence of the amplified DNA. Alternatively: (i) step (d) comprises contacting the mixture of (c) with a reverse primer downstream of the **deletion** sequence; (ii) to the mixture of (d), adding a forward primer complementary to the region upstream of the **deletion** sequence and each of 4 different nucleoside triphosphates as well as a DNA polymerase, under conditions so that only the **mutant** DNA is amplified; (iii) contacting the second aliquot with a forward primer complementary to a priming site within the **deletion** sequence, and a reverse primer downstream of the **deletion** sequence; (iv) to the mixture of (iii), adding 4 different nucleoside triphosphates, and a DNA polymerase under conditions so that the DNA is amplified; and (v) detecting the presence of the amplified DNA. Quantifying nucleic acids having **deletion** sequences comprises: (a) contacting an aliquot of sample comprising nucleic acids with **mutant** PCR primers under **short PCR** conditions; (b) amplifying the product; (c) contacting a different aliquot of the nucleic acid sample comprising nucleic acid with wild type PCR primers; (d) amplifying the

product; (e) identifying the presence of **amplicons**; and (f) quantifying the presence of **amplicons**. The aliquot is contacted with a cleavage reagent. The presence of **amplicons** is relative to the amount of wild type nucleic acid present in the sample. Quantification is conducted by comparison to a standard or by real-time monitoring. Preferred Kit: The kit further comprises **short PCR** reagents.

USE - The method is useful for detecting **mutant** nucleic acids, specifically a nucleic acid having a **deletion** (claimed).

EXAMPLE - A master mix comprising 10 mM Tris-HCl, 50 mM KCl, 5 mM MgCl2, Taq pol, and primer was subjected to a round of polymerase chain recation (PCR) at 92 degrees Centigrade for 15 seconds, and 71 degrees Centigrade for 45 seconds, for 40 cycles. No product gel bands were observed after subjecting the PCR products to agarose gel electrophoresis. The PCR profile used for the second round of amplification was 92 degrees Centigrade/15 seconds, 69 degrees Centigrade/45 seconds for 40 cycles. Gel bands of size compatible with deletions of the 5 kb region were seen in all samples examined using agarose gel electrophoresis. No primer-dimer bands were observed. One microliter of target DNA was removed from the PCR amplification that had been at an annealing-extension temperature of 71 degrees Centigrade, and was added to a reaction tube containing a new master mix. The following primers P1 and P2 were used, along with the beacon (B). The PCR condition of 92 degrees Centigrade/15 seconds, 71 degrees Centigrade/30 seconds for 10 cycles, 92 degrees Centigrade/15 seconds, 57 degrees Centigrade/10 seconds, and 69 degrees Centigrade/15 seconds for 30 cycles, was used. A fluorescent signal from the probe was monitored at 57 degrees Centigrade in real-time. Fluorescence versus cycle number was plotted for the 5 kb **mutant** sequence, in the presence and absence of target DNA. The increase in fluorescence, which crossed the threshold after PCR cycle approximately10, indicated a positive beacon signal for the 5 kb **deletion**. The fluorescent signal was consistent with the observation of the corresponding gel band described. Results of agarose gel electrophoresis showed the presence of a 5 kb **mutant** DNA in the sample as seen by a major band of approximate molecular weight of 174 base pairs (bp). 5' gccccaactaaatactaccg 3' (P1) 5' gatgtggtctttggagtagaaacctg 3' (P2) 5' TET-ccgctcgaaaggtattcctgctaatgctaggctgccaatcgagcgg-Dabcyl 3' (B)(45 pages)

L7 ANSWER 2 OF 3 MEDLINE on STN DUPLICATE 2

ACCESSION NUMBER: 1999393862 MEDLINE
DOCUMENT NUMBER: 99393862 PubMed ID: 10464596
TITLE: Accurate, high-throughput "snapshot" detection of hMLH1 mutations by two-dimensional DNA electrophoresis.
AUTHOR: Smith W M; Van Orsouw N J; Fox E A; Kolodner R D; Vijg J; Eng C
CORPORATE SOURCE: Charles A. Dana Human Cancer Genetics Unit, Dana-Farber Cancer Institute, Boston, MA 02115-6084, USA.
CONTRACT NUMBER: 1P30AG13314-02 (NIA)
SOURCE: GENETIC TESTING, (1998) 2 (1) 43-53.
Journal code: 9802546. ISSN: 1090-6576.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 19991026
Last Updated on STN: 19991026
Entered Medline: 19991012

AB The human genome and related projects have resulted in the isolation of a rapidly growing number of genes that cause susceptibility to human cancer. With rare exception, these genes are large and have disease-associated mutations scattered along the length of the genes. Therefore, the development of accurate and cost-efficient **mutation** detection

tests that can scan entire genes singly or in combination is warranted. hMLH1, encoding a mismatch repair enzyme, is a susceptibility gene for hereditary nonpolyposis colon cancer syndrome. This gene comprises 19 exons; mutations are scattered, typical of many susceptibility genes. Here, we present a strategy that combines extensive PCR multiplexing and two-dimensional DNA electrophoresis (Two-Dimensional Gene Scanning, TDGS) to scan accurately for mutations that lie within the exons and splice junctions of hMLH1. All target fragments, designed to have optimal melting characteristics, were prepared in a two-stage PCR--a four-plex long-distance PCR followed by **short PCR** in two multiplex groups of 10 and 11 **amplicons**. The mixture of **amplicons** was subjected to two-dimensional electrophoresis: separation by size in the first dimension and by melting characteristics in the second. Using this design, 41 samples containing known hMLH1 sequence variants or no alterations were blindly subjected to TDGS. All mutations were detected; there were no genuine false-positive or false-negative results. These results confirm that TDGS is a generally applicable, rapid, accurate, and reproducible **mutation** detection technology that would serve large-scale molecular epidemiologic studies as well as clinical molecular diagnostic purposes.

L7 ANSWER 3 OF 3 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 96372810 MEDLINE
DOCUMENT NUMBER: 96372810 PubMed ID: 8776589
TITLE: Mutational scanning of large genes by extensive PCR multiplexing and two-dimensional electrophoresis: application to the RB1 gene.
AUTHOR: Van Orsouw N J; Li D; van der Vlies P; Scheffer H; Eng C; Buys C H; Li F P; Vijg J
CORPORATE SOURCE: Department of Medicine, Beth Israel Hospital, Boston, MA 02215, USA.
CONTRACT NUMBER: EY05321 (NEI)
SOURCE: HUMAN MOLECULAR GENETICS, (1996 Jun) 5 (6) 755-61. Journal code: 9208958. ISSN: 0964-6906.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961205

AB With the rapid increase in the number of identified human disease genes, the development of accurate and cost-efficient **mutation** tests has become opportune. Here we present a combination of extensive PCR multiplexing and two-dimensional (2-D) DNA electrophoresis to screen for mutations in 26 exons of the retinoblastoma (RB1) tumor suppressor gene. In 2-D electrophoresis, fragments are separated according to size and base pair sequence in non-denaturing and denaturing gradient gels, respectively. All target fragments, designed to have optimal melting characteristics, were prepared in a two-step PCR (a 6-plex long-PCR pre-amplification and a subsequent 25-plex **short-PCR**) followed by heteroduplexing. The mixture of PCR **amplicons** was then subjected to 2-D electrophoresis under a single set of experimental conditions. With this design, 35 previously identified mutations in 18 different exons were detected in 33 bilateral retinoblastoma patients. These results suggest that 2-D electrophoresis in this format provides a generally applicable, practical and fast way to diagnose with high accuracy large genes for a broad spectrum of possible disease-causing mutations.

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PCR determination of inactivated RNA coliphage Qβ. Advances in Water a Wastewater Treatment Technology (2001), 101-108. Editor(s): Matsuo, Tomoi Publisher: Elsevier Science B.V., Amsterdam, Neth. CODEN: 69BVB4; Englis

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